

**ALLELES OF THE HUMAN KAPPA OPIOID RECEPTOR GENE, DIAGNOSTIC  
METHODS USING SAID ALLELES, AND METHODS OF TREATMENT BASED  
THEREON**

CROSS-REFERENCE TO RELATED APPLICATION

Priority 35 U.S.C. § 119(e) is claimed to U.S. provisional application serial no. 60/218,300, filed July 14, 2000, incorporated herein by reference in its entirety.

GOVERNMENTAL SUPPORT

This invention was made government support under Grant Nos. NIH-NIDA P50-DA05130, NIH-NIDA K05-DA00049, and NIH-NIDA R01-DA12848, awarded by the National Institute of Drug Addiction. The Government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates generally to alleles of the human kappa opioid receptor gene, polymorphisms thereof, methods of diagnosing various susceptibilities using such alleles and determining treatment for certain diseases based upon the presence of specific alleles, and various diseases or disorders related thereto.

BACKGROUND OF THE INVENTION

Opioid drugs have various effects on perception of pain, consciousness, motor control, mood, autonomic function, and can also induce physical dependence. The endogenous opioid system plays an important role in modulating endocrine, cardiovascular, respiratory, gastrointestinal functions, and immune functions. Opioids, either exogenous or endogenous, exert their actions by binding to specific membrane-associated receptors.

Examples of exogenous opioids presently known include, opium, heroin, morphine, codeine, fentanyl, and methadone, to name only a few. Moreover, a family of over 20 endogenous opioid peptides has been identified, wherein the members possess common structural features, including a positive charge juxtaposed with an aromatic ring that is required for interaction with an opioid receptor. It has been determined that most, if not all the endogenous opioid peptides are derived

1 from the proteolytic processing of three precursor proteins, i.e., pro-opiomelanocortin,  
2 proenkephalin, and prodynorphin. In addition, a fourth class of endogenous opioids, the  
3 endorphins, has been identified (the gene encoding these proteins has not yet been cloned). In  
4 the processing of the endogenous opioid precursor proteins, initial cleavages are made by  
5 membrane-bound proteases that cut next to pairs of positively charged amino acid residues, and  
6 then trimming reactions produce the final endogenous opioids secreted from cells *in vivo*.  
7 Different cell types contain different processing enzymes so that, for example  
8 proopiomelanocortin can be processed into different endogenous peptides by different cells. For  
9 example, in the anterior lobe of the pituitary gland, only corticotropin (ACTH),  $\beta$ -lipotropin, and  
10  $\beta$ -endorphin are produced. Both pro-enkephalin and pro-dynorphin are similarly processed by  
11 specific enzymes in specific cells to yield multiple opioid peptides.

12  
13 Pharmacological studies have suggested there are numerous classes of opioid receptors which  
14 bind to exogenous and endogenous opioids. These classes differ in their affinity for various  
15 opioid ligands and in their cellular and organ distribution. Moreover, although the different  
16 classes are believed to serve different physiological functions, there is substantial overlap of  
17 function, as well as of distribution.

18  
19 In particular, there are at least three known types of opioid receptors, mu ( $\mu$ ), delta ( $\delta$ ), and  
20 kappa ( $\kappa$ ), to which morphine, the enkephalins, and the dynorphins can bind. These three opioid  
21 receptor types are the sites of action of opioid ligands producing analgesic effects. However, the  
22 type of pain inhibited and the secondary functions vary with each receptor type. The mu  
23 receptor is generally regarded as primarily associated with pain relief, and drug or other  
24 chemical dependence, i.e., addiction and alcoholism.

25  
26 One such gene structurally related to the opioid receptor genes is the human kappa opioid  
27 (hKOR) receptor gene. The receptor is widely distributed in the CNS and periphery (including  
28 immune cells) and plays important and diverse roles in modulation of the endogenous opioid  
29 system, nociception, neurotransmitter release (including dopamine, GABA, and serotonin),  
30 learning, memory and cognition; cocaine, amphetamine and other stimulants self-administration;  
31 behavioral sensitization to cocaine, opiates, alcohol and tobacco; opiate, amphetamine and  
32 alcohol withdrawal, physical dependence and tolerance; neuroendocrine function, reproductive  
33 function, prolactin regulation, stress responsivity; physiology and pathology of mood and affect;

immune function, and gastrointestinal function. See, for example, Simonin F, Valverde O, Smadja C, Slowe S, Kitchen I, Dierich A, Le Meur M, Roques BP, Maldonado R, Kieffer BL, 1998, Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50,488H and attenuates morphine withdrawal, *EMBO J.*, **17**: 886-897; Slowe S, Simonin F, Kieffer B, Kitchen I. 1999, Quantitative autoradiography of  $\mu$ -,  $\delta$ - and  $\kappa$ -opioid receptors in  $\kappa$ -opioid receptor knockout mice, *Brain research*, **818**: 335-345; Heidbreder CA, Schenk S, Partridge B, Shippenberg TS. 1998, Increased responsiveness of mesolimbic and mesostriatal dopamine neurons to cocaine following repeated administration of a selective kappa-opioid receptor agonist, *Synapse*, **30**: 255-262; Schenk S, Partridge B, Shippenberg TS. 1999, U69593, a kappa-opioid agonist, decreases cocaine self-administration and decreases cocaine-produced drug-seeking, *Psychopharmacology (Berl)*, **144**: 339-346; Kreek MJ, Schluger J, Borg L, Gunduz M, Ho A. 1999, Dynorphin A1-13 causes elevation of serum levels of prolactin through an opioid receptor mechanism in humans: gender differences and implications for modulation of dopaminergic tone in the treatment of addictions. *JPET*, **288**: 260-269; Portenoy R, Caraceni A, Cherny NI, Goldblum R, Ingham J, Inturrisi CE, Johnson JH, Lapin J, Tiseo PJ, Kreek MJ. 1999, Dynorphin A(1-13) analgesia in opioid-treated patients with chronic pain. *Clin Drug Invest.*, **17**: 33-42; Milan MJ. 1990,  $\kappa$ -Opioid receptors and analgesia. *TIPS*, **11**: 70-76; Mansson E, Bare L, Yang D., 1994, Isolation of human  $\kappa$  opioid receptor cDNA from placenta, *Bioch Biophys Res Communications*, **202**, 1431-1437; Simonin F, Gaveriaux-Ruff C, Befort K, Matthes H, Iannas B, Micheletti G, Mattei M-G, Charron G, Bloch B, Kieffer B., 1995,  $\kappa$ -Opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system, *Proc Natl Acad Sci USA*, **92**, 7006-7010; Zhu J, Chen C, Xue J-C, Kunapuli S, DeRiel JK, Liu-Chen L-Y., 1995, Cloning of a human  $\kappa$  opioid receptor from the brain, *Life Sciences*, **56**, 201-207; Grandy DK., 1994, Mapping of the human kappa opioid receptor gene to chromosome 8q11.2-q12: no evidence for multiple kappa opioid receptor genes (partial sequence of exon II and downstream intron). GenBank entry, Accession # U16860; and Yasuda K, Espinosa R, Takeda J, Le Beau MM, Bell GL., 1995, Localization of kappa opioid receptor gene to human chromosome band 8q11.2 (sequence of exon II), GenBank entry, Accession # L26079. Three GenBank entries for hKOR are U17298, NM\_000912, and L37362. These as well as all publications cited herein are incorporated herein by reference in their entireties.

1 It is toward the identification of both the wild-type human kappa opioid receptor gene as well as  
2 alleles other than the most common or wild-type allele of the human kappa opioid receptor gene,  
3 polymorphisms therein, and combinations of such polymorphisms that can be used as genetic  
4 markers to map the locus of the human kappa opioid receptor gene in the genome, and  
5 additionally to correlate such polymorphisms of the human kappa opioid receptor gene with  
6 susceptibility of a subject to any of the various physiological functions, conditions and diseases  
7 mentioned hereinabove in which the kappa opioid receptor gene plays a role, including but not  
8 limited to determine a subject's increased or decreased susceptibility to addictive diseases,  
9 susceptibility to pain and response to analgesics, physiological responses related to the  
10 endogenous opioid system, nociception, neurotransmitter release (including dopamine, GABA,  
11 and serotonin), learning, memory and cognition; cocaine, amphetamine and other stimulants  
12 self-administration; behavioral sensitization to cocaine, opiates, alcohol and tobacco; opiate,  
13 amphetamine and alcohol withdrawal, physical dependence and tolerance; neuroendocrine  
14 function, reproductive function, prolactin regulation, stress responsivity; physiology and  
15 pathology of mood and affect; immune function, and gastrointestinal function; among other uses,  
16 that the present invention is directed.

17  
18 The citation of any reference herein should not be construed as an admission that such reference  
19 is available as "Prior Art" to the instant application.  
20

#### 21 SUMMARY OF THE INVENTION

22 There is provided, in accordance with the present invention, heretofore unknown single-  
23 nucleotide polymorphisms (SNPs) of the human kappa opioid receptor gene, and their use in  
24 mapping the locus of the human kappa opioid receptor gene; determining susceptibility to  
25 addictive diseases; determining susceptibility to pain; determining a therapeutically effective  
26 amount of pain reliever to administer to a subject suffering from pain; diagnosing a disease or  
27 disorder in a subject related to a physiological response, condition or disorder such as but not  
28 limited to the endogenous opioid system, nociception, neurotransmitter release (including  
29 dopamine, GABA, and serotonin), learning, memory and cognition; cocaine, amphetamine and  
30 other stimulants self-administration; behavioral sensitization to cocaine, opiates, alcohol and  
31 tobacco; opiate, amphetamine and alcohol withdrawal, physical dependence and tolerance;  
32 neuroendocrine function, reproductive function, prolactin regulation, stress responsivity;  
33 physiology and pathology of mood and affect; immune function, and gastrointestinal function;

1 and selecting an appropriate therapeutic agent and a therapeutically effective amount of such an  
2 agent to administer to a subject suffering from an aforementioned disease or disorder. One or  
3 more of the polymorphisms of the invention may be employed as such; and an individual may  
4 have one or more of the polymorphisms. Moreover, the polymorphisms individually and in  
5 combination may be present homozygously or heterozygously.

6  
7 The single-nucleotide polymorphisms identified herein are present in exon III of the hKOR gene:  
8 C852T (SEQ ID No:2), present in transmembrane region (TM) VI; C948T (SEQ ID No:3),  
9 present in TM VII; and C1008T (SEQ ID No:4), present in the C-terminal region of exon III.

10  
11 In addition, by comparing the published hKOR GenBank sequences mentioned above with the  
12 sequences of the KOR of numerous subjects in the study described hereinbelow, the present  
13 inventors have identified the most common, or wild-type, allele of hKOR (SEQ ID No:1) and  
14 determined that variations therein which are present in the aforementioned GenBank sequences  
15 are indeed single nucleotide polymorphisms (SNPs) of hKOR, in particular, those in  
16 NM\_000912 as compared with U17298 or L37362. These polymorphisms are G36T (SEQ ID  
17 No:5), present in the N-terminal portion of exon I of hKOR; and in exon III, the polymorphisms  
18 A843G (SEQ ID No:6), present in TM VI; and C846T (SEQ ID No:7), present in TM VI. All of  
19 these SNPs are silent, i.e., they do not alter the predicted amino acid sequence of the encoded  
20 receptor protein.

21  
22 Thus, in summary, the polymorphisms identified herein in the human kappa opioid receptor are  
23 C852T (SEQ ID No:2), C948T (SEQ ID No:3), C1008T (SEQ ID No:4), G36T (SEQ ID No:5),  
24 A843G (SEQ ID No:6), and C846T (SEQ ID No:7). The wild-type or most common allele has  
25 been identified herein as that depicted in SEQ ID No:1.

26  
27 The present invention extends to DNA sequences of heretofore unknown isolated nucleic acid  
28 molecules which encode human kappa opioid receptors, wherein the DNA sequences include any  
29 combination of the aforementioned known polymorphisms.

30  
31 The present invention further extends to diagnostic methods to determine a subject's increased or  
32 decreased susceptibility to addictive diseases. With the results of such methods, targeted  
33 prevention methods, early therapeutic intervention, and improved chronic treatment to opioid

addiction are set forth herein and encompassed by the present invention. In addition, attending medical professionals armed with the results of such diagnostic methods can determine whether administration of opioid analgesics is appropriate or whether non-opioid derived analgesics should be administered to the subject. Furthermore, appropriate choice and type of analgesic to treat a subject's pain can be made. Such determination may be made by identification of any individual or any combination of the above-mentioned polymorphisms, using such non-limiting methods as DNA sequencing, differential hybridization to biological chip arrays such as an oligonucleotide gelpad microchip, or single nucleotide extension (SNE) on chip arrays such as on oligonucleotide gelpad microchips.

Broadly the present invention extends to an isolated variant allele of a human kappa opioid receptor gene which can serve as a genetic marker, wherein the predominant or "most common" allele of a human kappa opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises G36T, A843G, C846T, C852T, C948T, C1008T, or any combination thereof.

Furthermore, the present invention extends to an isolated variant allele of a human kappa opioid receptor gene as set forth above, which is detectably labeled. Numerous detectable labels have applications in the present invention, such as radioactive elements, chemicals which fluoresces, or enzymes, to name only a few.

The present invention further extends to an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of the human kappa opioid receptor gene, wherein the predominant or "most common" allele of a human kappa opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises G36T, A843G, C846T, C852T, C948T, C1008T, or any combination thereof.

Moreover, the present invention extends to an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of the human kappa opioid receptor gene, wherein the predominant or "most common" allele of a human kappa opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present

1 invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation  
2 comprises G-46A, G36T, A843G, C846T, C852T, C948T, C1008T, or any combination thereof,  
3 wherein the isolated nucleic acid molecule is detectably labeled. Examples of detectable labels  
4 that have applications in this embodiment of the present invention are described above.

5  
6 In addition, the present invention extends to cloning vectors that can be used to clone copies of a  
7 variant alleles of a human kappa opioid receptor gene of the present invention. For example, the  
8 present invention extends to a cloning vector comprising an isolated variant allele of a human  
9 kappa opioid receptor gene and an origin of replication, wherein the predominant or "most  
10 common" allele of a human kappa opioid receptor gene found in the population comprises a  
11 DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA  
12 sequence having a variation in SEQ ID NO:1, wherein the variation comprises G36T, A843G,  
13 C846T, C852T, C948T, C1008T, or any combination thereof.

14  
15 In another embodiment, the present invention extends to a cloning vector comprising an isolated  
16 nucleic acid molecule selectively hybridizable to an isolated variant allele of a human kappa  
17 opioid receptor gene, and an origin of replication, wherein the predominant or "most common"  
18 allele of a human kappa opioid receptor gene found in the population comprises a DNA sequence  
19 of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having  
20 a variation in SEQ ID NO:1, wherein the variation comprises G36T, A843G, C846T, C852T,  
21 C948T, or C1008T, or any combination thereof.

22  
23 Numerous cloning vectors have applications in the present invention. For example, a cloning  
24 vector having applications in the present invention includes *E. coli*, bacteriophages such as  
25 lambda derivatives, plasmids such as pBR322 derivatives, and pUC plasmid derivatives such as  
26 pGEX vectors or pmal-c or pFLAG, to name only a few.

27  
28 Naturally, the present invention extends to expression vectors comprising an isolated variant  
29 allele a human kappa opioid receptor gene operatively associated with a promoter, wherein the  
30 predominant or "most common" allele of a human kappa opioid receptor gene found in the  
31 population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present  
32 invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation  
33 comprises G36T, A843G, C846T, C852T, C948T, or C1008T, or any combination thereof.

1 Furthermore, the present invention extends to an expression vector comprising an isolated  
2 nucleic acid molecule selectively hybridizable to an isolated variant allele a human kappa opioid  
3 receptor gene, wherein the isolated nucleic acid molecule is operatively associated with a  
4 promoter. As set forth above, the predominant or "most common" allele of a human kappa  
5 opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a  
6 variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID  
7 NO:1, wherein the variation comprises G36T, A843G, C846T, C852T, C948T, or C1008T, or  
8 any combination thereof.

9  
10 Numerous promoters have applications in an expression vector of the present invention,  
11 including but not limited to immediate early promoters of hCMV, early promoters of SV40,  
12 early promoters of adenovirus, early promoters of vaccinia, early promoters of polyoma, late  
13 promoters of SV40, late promoters of adenovirus, late promoters of vaccinia, late promoters of  
14 polyoma, the *lac* the *trp* system, the *TAC* system, the *TRC* system, the major operator and  
15 promoter regions of phage lambda, control regions of fd coat protein, 3-phosphoglycerate kinase  
16 promoter, acid phosphatase promoter, or promoters of yeast  $\alpha$  mating factor, to name only a few.

17  
18 In addition, the present invention extends to a unicellular host transformed or transfected with an  
19 expression vector of the present invention. Examples of hosts which can be transformed or  
20 transfected with an expression vector of the present invention, and have applications in the  
21 present invention, include, but are not limited to, *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*,  
22 yeast, CHO, R1.1, B-W, L-M, COS1, COS7, BSC1, BSC40, BMT10 or Sf9 cells.

23  
24 The invention further extends to altered expression of the wild-type kappa opioid gene product,  
25 and means for detecting the altered expression, as a consequence of the presence of any one or  
26 any combination of the polymorphisms G36T, A843G, C846T, C852T, C948T, or C1008T.

27  
28 Accordingly, the present invention extends to a method for determining a susceptibility in a  
29 subject to at least one disease, comprising the steps of removing a bodily sample comprising a  
30 first and second allele of a human kappa opioid receptor gene from the subject, and determining  
31 whether the first allele comprises a human kappa opioid receptor gene comprising a DNA  
32 sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises G36T,  
33 A843G, C846T, C852T, C948T, or C1008T.



1 The present of at least one of these variations in the human kappa opioid receptor gene of the  
2 first allele is expected to be indicative of the subject's susceptibility to at least one disease  
3 relative to the susceptibility of a standard, wherein the standard comprises a first allele  
4 comprising a human kappa opioid receptor gene having a DNA sequence of SEQ ID NO:1.

5  
6 Another embodiment of the method for determining a susceptibility in the subject to at least one  
7 disease, as described above, comprises the further step of determining whether the second allele  
8 of the bodily sample of the subject comprises a human kappa opioid receptor gene comprising a  
9 DNA sequence having at least one variation in SEQ ID NO:1, wherein the variations comprise  
10 G36T, A843G, C846T, C852T, C948T, or C1008T.

11  
12 Furthermore, the present invention extends to a method for determining a susceptibility to pain  
13 in a subject relative to susceptibility to pain in a standard, comprising the steps of removing a  
14 bodily sample comprising a first and second allele of a human kappa opioid receptor gene from  
15 the subject, and determining whether the first allele comprises a human kappa opioid receptor  
16 gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the  
17 variation comprises one or more of the polymorphisms G36T, A843G, C846T, C852T, C948T,  
18 or C1008T. The presence of at least one variation in the human kappa opioid receptor gene of  
19 the first allele is expected to be indicative of a decreased or increased susceptibility to pain in the  
20 subject relative to susceptibility to pain in the standard, wherein the first allele of the standard  
21 comprises a human kappa opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

22  
23 Moreover, a method for determining a susceptibility to pain in a subject may further comprise  
24 the step of determining whether the second allele comprises a human kappa opioid receptor gene  
25 comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation  
26 comprises one or more of the polymorphisms G36T, A843G, C846T, C852T, C948T, or  
27 C1008T. The presence of the at least one variation in the human kappa opioid receptor gene of  
28 the second allele of the bodily sample from the subject is expected to be indicative of an  
29 increased or decreased susceptibility to pain in the subject relative to the susceptibility to pain in  
30 the standard, wherein the second allele in the standard comprises a human kappa opioid receptor  
31 gene comprising a DNA sequence of SEQ ID NO:1.

32  
33 Consequently, the present invention extends to a method for determining a therapeutically

1 effective amount of pain reliever to administer to a subject in order to induce analgesia in the  
2 subject relative to a therapeutically effective amount of the pain reliever to administer to a  
3 standard in order to induce analgesia in the standard, wherein the method comprises determining  
4 a susceptibility to pain in the subject relative to susceptibility to pain in the standard. The  
5 susceptibility of pain in the subject is expected to be indicative of the therapeutically effective  
6 amount of the pain reliever to administer to the subject to induce analgesia in the subject relative  
7 to the amount of the pain reliever to administer to the standard to induce analgesia in the  
8 standard.

9  
10 Hence, the present invention extends to a method for determining a therapeutically effective  
11 amount of pain reliever to administer to a subject in order to induce analgesia in the subject  
12 relative to a therapeutically effective amount of the pain reliever to administer to a standard in  
13 order to induce analgesia in the standard wherein the method comprises the steps of removing a  
14 bodily sample comprising a first and second allele of a human kappa opioid receptor gene from  
15 the subject, and determining whether the first allele comprises a human kappa opioid receptor  
16 gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the at  
17 least one variation comprises G36T, A843G, C846T, C852T, C948T, or C1008T. The presence  
18 of at least one variation in the human kappa opioid receptor gene of the first allele from the  
19 bodily sample is expected to be indicative of the therapeutically effective amount of pain reliever  
20 to administer to the subject to induce analgesia in the subject relative to the therapeutically  
21 effective amount of pain reliever to administer to the standard to induce analgesia in the  
22 standard, wherein the standard comprises a first allele comprising a human kappa opioid receptor  
23 gene comprising a DNA sequence of SEQ ID NO:1.

24  
25 Moreover, the present invention further extends to a method for determining a therapeutically  
26 effective amount of pain reliever to administer to a subject in order to induce analgesia in the  
27 subject relative to a therapeutically effective amount of pain reliever to administer to a standard  
28 to induce analgesia therein, further comprising the steps of removing a bodily sample comprising  
29 a first and second allele comprising a human kappa opioid receptor gene from the subject, and  
30 determining whether the second allele of the bodily sample comprises a human kappa opioid  
31 receptor gene comprising a DNA sequence comprising at least one variation in SEQ ID NO:1,  
32 wherein the at least one variation comprises G36T, A843G, C846T, C852T, C948T, or C1008T.  
33 The presence of at least one variation in the human kappa opioid receptor gene of the first and/or

1 second allele of the bodily sample is expected to be indicative of the therapeutically effective  
2 amount of pain reliever to administer to the subject to induce analgesia therein relative to the  
3 amount of pain reliever to administer to a standard to induce analgesia therein, wherein the first  
4 and second alleles of the standard comprise a human kappa opioid receptor gene comprising a  
5 DNA sequence of SEQ ID NO:1.

6  
7 Examples of pain relievers having applications in this embodiment of the present invention  
8 include, but are not limited to, morphine, codeine, dihydromorphan, meperidine, methadone,  
9 fentanyl and its congeners, butorphenol, nalbuphine, LAAM, or propoxyphene, to name only a  
10 few.

11  
12 The present invention further extends to commercial test kits suitable for use by a medical  
13 professional to determine whether either or both alleles of a bodily sample taken from a subject  
14 comprise a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation  
15 comprises G36T, A843G, C846T, C852T, C948T, or C1008T.

16  
17 Commercial test kits of the present invention have applications in determining susceptibility of  
18 pain in the subject relative to a standard. Such kits can also be used to determine a subject's  
19 increased or decreased susceptibility to at least one addictive disease relative to susceptibility to  
20 at least one addictive disease in a standard. Also a therapeutically effective amount of pain  
21 reliever to administer to the subject in order to induce analgesia in the subject relative to a  
22 therapeutically effective amount of pain reliever to administer to a standard to induce analgesia  
23 in the standard can be determined. Moreover, a test kit of the present invention has applications  
24 in determining a therapeutically effective amount of therapeutic agent for treating at least one  
25 physiological response, condition or disease to administer to a subject suffering therefrom,  
26 relative to a therapeutically effective amount of therapeutic agent to administer to a standard.

27  
28 Furthermore, a commercial test kit of the present invention can also be used to determine the  
29 presence of an isolated variant allele of a human kappa opioid receptor gene of the present  
30 invention in a bodily sample removed from a subject, which can serve as a genetic marker. As  
31 explained above, the predominant or "most common" allele of a human kappa opioid receptor  
32 gene found in the population comprises a DNA sequence of SEQ ID NO:1. Hence a variant  
33 allele comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation

comprises G36T, A843G, C846T, C852T, C948T, or C1008T, or combinations thereof, can be detected in the bodily sample with a commercial kit of the invention.

Accordingly, a commercial test kit may be prepared for determining the presence of at least one variation in a human kappa opioid receptor gene of either or both alleles in a bodily sample taken from a subject, wherein the commercial test kit comprises:

- a) PCR oligonucleotide primers suitable for detection of an allele comprising a human kappa opioid receptor gene having a DNA sequence with a variation in SEQ ID NO:1;
- b) other reagents; and
- c) directions for use of the kit.

Accordingly, the present invention extends to a commercial test kit having applications set forth above, comprising a predetermined amount of at least one detectably labeled immunochemically reactive component having affinity for a variant human kappa opioid receptor;

- (b) other reagents; and
- (c) directions for use of the kit.

In a further variation, the test kit may be prepared and used for the purposes stated above, which operates according to a predetermined protocol (e.g. "competitive," "sandwich," "double antibody," etc.), and comprises:

- (a) a labeled component which has been obtained by coupling the human kappa opioid receptor of a bodily sample to a detectable label;
- (b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand comprises:
  - (i) a ligand capable of binding with the labeled component (a);
  - (ii) a ligand capable of binding with a binding partner of the labeled component (a);
  - (iii) a ligand capable of binding with at least one of the component(s) to be determined; or
  - (iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; or
- (c) directions for the performance of a protocol for the detection and/or determination of one

1 or more components of an immunochemical reaction between the human kappa opioid  
2 receptor gene of the present invention and a specific binding partner thereto.

3  
4 The present invention is also directed to the finding of the most common, or wild-type, human  
5 kappa opioid receptor gene sequence, as depicted in SEQ ID No:1.

6  
7 Accordingly, it is an object of the present invention to provide heretofore unknown variations the  
8 DNA sequence of the human kappa opioid receptor gene wherein the variations can be used to  
9 map the locus of the human kappa opioid receptor gene.

10  
11 It is yet another object of the present invention to use heretofore unknown polymorphisms of an  
12 allele of the human kappa opioid receptor gene as markers for any kind of disorder related to the  
13 human kappa opioid receptor, such as an addictive disease, pain, or markers for genes.

14  
15 It is another object of the present invention to provide nucleotides, optionally detectably labeled,  
16 selectively hybridizable to variant alleles of the human kappa opioid receptor gene disclosed  
17 herein, as well as polypeptides produced from the expression of the variant alleles and  
18 nucleotides selectively hybridizable thereto under selective hybridization conditions.

19  
20 It is another object of the present invention to gain insight into a subject's susceptibility to pain.  
21 This insight can be used to determine a therapeutically effective dose of pain reliever to  
22 administer to the subject to induce analgesia therein relative to the therapeutically effective  
23 amount of pain reliever administered to a standard to induce analgesia therein, wherein the  
24 standard comprises two alleles of the human kappa opioid receptor gene comprising a DNA  
25 sequence of SEQ ID NO:1.

26  
27 Such information can be used to tailor a regimen for treating a subject suffering from at least one  
28 addictive disease, relative to the therapeutically effective amount of therapeutic agent  
29 administered to a standard suffering from at least one addictive disease.

30  
31 It is yet another object of the present invention to provide commercial test kits for attending  
32 medical professionals to determine the presence of variant alleles of a human kappa opioid  
33 receptor gene in a bodily sample taken from a subject. The results of such testing can then be

1 used to determine the subject's nociception, neurotransmitter release (including dopamine,  
2 GABA, noradrenaline, and serotonin), learning, memory and cognition; cocaine, amphetamine  
3 and other stimulants self-administration; behavioral sensitization to cocaine, opiates, alcohol and  
4 tobacco; opiate, amphetamine and alcohol withdrawal, physical dependence and tolerance;  
5 neuroendocrine function, reproductive function, prolactin regulation, stress responsivity;  
6 physiology and pathology of mood and affect; immune function, and gastrointestinal function;  
7 determining a therapeutically effective amount of pain reliever to administer to the subject in  
8 order to induce analgesia, or determining a therapeutically effective amount of therapeutic agent  
9 for treating at least one addictive disease to administer to the subject.

10  
11 It is yet another object of the present invention to provide commercial detecting variant alleles of  
12 the human kappa opioid receptor gene or the presence of a variant human kappa opioid receptor  
13 in a bodily sample taken from a subject. The results of such tests can then be used to gain incite  
14 into a subject's ability to withstand pain, susceptibility to addiction, to diagnose a disease or  
15 disorder related to nociception, neurotransmitter release (including dopamine, GABA,  
16 noradrenaline, and serotonin), learning, memory and cognition; cocaine, amphetamine and other  
17 stimulants self-administration; behavioral sensitization to cocaine, opiates, alcohol and tobacco;  
18 opiate, amphetamine and alcohol withdrawal, physical dependence and tolerance;  
19 neuroendocrine function, reproductive function, prolactin regulation, stress responsivity;  
20 physiology and pathology of mood and affect; immune function, and gastrointestinal function.

21  
22 These and other aspects of the present invention will be better appreciated by reference to the  
23 following drawings and Detailed Description.

#### 24 25 BRIEF DESCRIPTION OF THE DRAWINGS

26 **Figure 1** depicts the nucleic acid sequence of the most common allele (wild type) of the human  
27 kappa opioid receptor gene (SEQ ID NO:1).

28  
29 **Figure 2** depicts the nucleic acid sequence of the C852T polymorphism in exon III of the human  
30 kappa opioid receptor gene (SEQ ID NO:2).

31  
32 **Figure 3** depicts the nucleic acid sequence of the C948T polymorphism in exon III of the human  
33 kappa opioid receptor (SEQ ID NO:3).

1  
2 **Figure 4** depicts the nucleic acid sequence of the C1008T polymorphism in exon III of the  
3 human kappa opioid receptor (SEQ ID NO:4).  
4

5 **Figure 5** depicts the nucleic acid sequence of the G36T polymorphism in exon I of the human  
6 kappa opioid receptor gene (SEQ ID NO:5).  
7

8 **Figure 6** depicts the nucleic acid sequence of the A843G polymorphism in exon III of the human  
9 kappa opioid receptor gene (SEQ ID NO:6).  
10

11 **Figure 7** depicts the nucleic acid sequence of the C846T polymorphism in exon III of the human  
12 kappa opioid receptor gene (SEQ ID NO:7).  
13

#### 14 DETAILED DESCRIPTION OF THE INVENTION

15 As explained above, the present invention is based upon Applicants' surprising and unexpected  
16 discovery of heretofore unknown single nucleotide polymorphisms (SNPs) in the human kappa  
17 opioid receptor (hKOR) gene, along with combinations thereof. Polymorphisms in this gene  
18 have not been previously recognized or known. Furthermore, Applicants have identified the  
19 most common, or wild-type allele, of the hKOR, SEQ ID No:1, based on sequencing hKOR  
20 genes from a large number of individuals, and have identified in one of the previously-known  
21 hKOR sequences as a variant thereof, comprising three polymorphisms. In addition, the  
22 inventors herein have discovered that more than one polymorphism can be present in either or  
23 both alleles of the human kappa opioid receptor gene in a subject.  
24

25 In addition, the present invention is based upon Applicants' surprising discovery of molecules of  
26 heretofore unknown isolated nucleic acid molecules which encode human kappa opioid  
27 receptors, wherein the DNA sequences comprise one or more polymorphisms as set forth herein.  
28

29 Furthermore, the present invention is based upon Applicants' surprising and unexpected  
30 discovery that the expression of variant alleles of the human kappa opioid receptor gene  
31 comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variations are  
32 C852T (SEQ ID No:2), present in transmembrane region (TM) VI of exon III; C948T (SEQ ID  
33 No:3), present in TM VII of exon III; C1008T (SEQ ID No:4), present in the C-terminal region

1 of exon III; G36T (SEQ ID No:5), present in the N-terminal portion of exon I; A843G (SEQ ID  
2 No:6), present in TM VI of exon III; and C846T (SEQ ID No:7), present in TM VI of exon III.

3  
4 The present invention further extends to heretofore unknown polymorphisms of the human kappa  
5 opioid receptor gene that can serve as genetic markers to map the locus of the human kappa  
6 opioid receptor gene.

7  
8 As noted above, the human kappa opioid receptor plays important and diverse roles in  
9 modulation of the endogenous opioid system, nociception, neurotransmitter release (including  
10 dopamine, GABA, and serotonin), learning, memory and cognition; cocaine, amphetamine and  
11 other stimulants self-administration; behavioral sensitization to cocaine, opiates, alcohol and  
12 tobacco; opiate, amphetamine and alcohol withdrawal, physical dependence and tolerance;  
13 neuroendocrine function, reproductive function, prolactin regulation, stress responsivity;  
14 physiology and pathology of mood and affect; immune function, and gastrointestinal function.  
15 As noted herein, reference to the identification of one or more of the polymorphisms described  
16 herein and the relationship to physiological response, conditions, disorders, diseases,  
17 pathologies, aberrations, and other variations in normal or pathological states relating to the  
18 aforementioned physiologic processes is embraced herein as utilities for which the identification  
19 of the polymorphisms may be applied. Moreover, the identification of the polymorphisms,  
20 whether heterozygous, homozygous, single or multiple polymorphisms in an individual and the  
21 linkage of such single or multiple polymorphisms, homozygous or heterozygous, to  
22 susceptibility, propensity, therapeutic potential, and other factors are further embraced herein.  
23

24 The present invention extends to diagnostic methods to determine a subject's increased or  
25 decreased susceptibility to at least one disease, including addictive disease. With the results of  
26 such methods, targeted prevention methods, early therapeutic intervention, and improved chronic  
27 treatment to opioid addiction are set forth herein and encompassed by the present invention. In  
28 addition, attending medical professionals of subjects armed with the results of such diagnostic  
29 methods can determine whether administration of opioid analgesics is appropriate or whether  
30 non-opioid derived analgesics should be administered to the subject. Also, appropriate choice  
31 and type of analgesic can be made in treating a subject's pain.

32  
33 Methods for determining the presence of the one or more polymorphisms may be made using



any of a large variety of methods for identifying altered nucleotides present in a nucleic acid sequence, by way of non-limiting examples as conventional DNA sequencing, differential hybridization to biological chip arrays such as an oligonucleotide gelpad microchip, or single nucleotide extension (SNE) on chip arrays such as on oligonucleotide gelpad microchips. These methods are known to one of skill in the art, and are merely exemplified by the following citations: Khrapko KR, Lysov YP, Khorlin A, Shick VV, Florentiev VL, Mirzabekov AD. 1989. An oligonucleotide hybridization approach to DNA sequencing. *FEBS Lett* 256:118-122; Khrapko KR, Lysov YP, Khorlin AA, Ivanov IB, Yershov GM, Vasilenko SL, Florentiev V, Mirzabekov AD, 1991, A method for DNA sequencing by hybridization with oligonucleotide matrix. *J DNA sequencing* 1: 375-388; Fodor SPA, Read JL, Pirrung MC, Stryer L, Lu AT, Solas, D, 1991, Light directed, spatially addressable parallel chemical synthesis. *Science* 251:776-773; Southern EM, Maskos U, Elder JK, 1992, Analyzing and comparing nucleic acid sequences by hybridization to arrays of oligonucleotides: evaluation using experimental models, *Genomics* 13:1008-1017; Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SPA. 1996. Accessing genetic information with high-density DNA arrays. *Science* 274:610-614; Hacia JG, Brody LC, Chee MS, Fodor SPA, Collins F. 1996. Detection of heterozygous mutations in BCRA1 using high density oligonucleotide arrays and two colour fluorescence analysis. *Nature Genet* 14:44-447; Yershov G, Barsky V, Belgovskiy A, Kirillov E, Kreindlin E, Ivanov I, Parinov S, Guschin D, Drobishev A, Dubiley S, Mirzabekov A. 1996. DNA Analysis and diagnostics on oligonucleotide microchips. *Proc Natl Acad Sci USA* 93:4913-4918; Shick VV Lebed YB, Kryukov GV. 1998. Identification of HLA DQA1 alleles by the oligonucleotide microchip method. *Mol Biol* 32:697-688. Translated from *Molekulyarna Biologiya* 32:813-822; Wang DG, Fan J-B, Siao C-J, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipschutz R, Chee M, Lander ES. 1998 Large scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 280:1077-1082; Halushka MK, Fan J-B, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, Chakravarti A. 1999. Patterns of single-nucleotide polymorphisms in candidate genes for blood pressure homeostasis. *Nature Genet* 22:239-247; Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES. 1999. Characterization of single nucleotide polymorphisms in coding regions of human genes. *Nature genet* 22:231-238; Parinov S, Barsky

1 V, Yershov G, Kirillov E, Timofeev E, Belgovskiy A, Mirzabekov A. 1996. DNA sequencing by  
2 hybridization to microchip octa- and decanucleotides extended by stacked pentanucleotides.  
3 Nucleic Acids Res 24:2998-3004; Guschin D, Yershov G, Zaslavsky A, Gemmell A, Shick V,  
4 Proudnikov V, Arenkov P, Mirzabekov A. 1997. Manual manufacturing of oligonucleotide,  
5 DNA and protein microchips. Anal Biochem 250:203-211; Drobyshev A, Mologina M. Shik V,  
6 Pobedinskaya D, Yershov G, Mirzabekov A. 1997. Sequence analysis by hybridization with  
7 oligonucleotide microchip: Identification of b-thalassemia mutations. Gene 188:45-52; Syvänen  
8 A-C, Aalto-Setälä K, Harju L, Kontula K, SØderlund H. 1990. A primer-guided nucleotide  
9 incorporation assay in the genotyping of apolipoprotein E. Genomics 8:684-692; Pastinen T,  
10 Kurg A, Metspalu A, Peltonen L, Syvänen A-C. 1997. Minisequencing: A specific tool for DNA  
11 analysis and diagnostics on oligonucleotide arrays. Genome res 7:606-614; Pastinen T, Perola M,  
12 Niini P, Terwilliger J, Salomaa V, Vartiainen E, Peltonen L, Syvänen A-C. 1998. Array-based  
13 multiplex analysis of candidate gene reveals two independent and additive genetic risk factors  
14 for myocardial infarction in the Finnish population. Hum Mol Genet 7:1453-1462; Dubiley S,  
15 Kirillov E, Mirzabekov A. 1999. Polymorphism analysis and gene detection by minisequencing  
16 on an array of gel-immobilized primers. Nucleic Acids Res 27:e19; and Syvänen A-C. 1999.  
17 From gels to chips: "Minisequencing" primer extension analysis of point mutations and single  
18 nucleotide polymorphisms. Hum Mutat 13:1-10. Such citations are not intended to be limiting  
19 but merely exemplary of the various methods available for detecting one or more of the  
20 polymorphisms described herein.

21  
22 Also, the present invention extends to methods of determining a subject's increased or decreased  
23 susceptibility to pain and response to analgesics, and using that information when prescribing  
24 analgesics to the subject.

25  
26 The present invention further extends to variant alleles of the human kappa opioid receptor gene  
27 comprising a DNA sequence comprising one or more heretofore unknown polymorphisms,  
28 G36T, A843G, C846T, C852T, C948T, or C1008T.

29  
30 Consequently, an initial aspect of the present invention involves isolation of heretofore unknown  
31 variant alleles of the human kappa opioid receptor gene. As used herein, the term "gene" refers  
32 to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA  
33 nucleic acids.

Furthermore, in accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And Translation* [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; *Immobilized Cells And Enzymes* [IRL Press, (1986)]; B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*, i.e., capable of replication under its own control.

A "cassette" refers to a segment of DNA that can be inserted into a vector at specific restriction sites. The segment of DNA encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation.

A cell has been "transfected" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous DNA when the transfected DNA effects a phenotypic change. Preferably, the transforming DNA should be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

"Heterologous" DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

1 A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides  
2 (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides  
3 (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or  
4 any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single  
5 stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-  
6 RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA  
7 molecule, refers only to the primary and secondary structure of the molecule, and does not limit  
8 it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter*  
9 *alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and  
10 chromosomes. In discussing the structure of particular double-stranded DNA molecules,  
11 sequences may be described herein according to the normal convention of giving only the  
12 sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having  
13 a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule  
14 that has undergone a molecular biological manipulation.

15  
16 A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA,  
17 genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to  
18 the other nucleic acid molecule under the appropriate conditions of temperature and solution  
19 ionic strength (*see* Sambrook et al., *supra*). The conditions of temperature and ionic strength  
20 determine the "stringency" of the hybridization. Polynucleotides capable of discriminating  
21 between the wild-type and polymorphic alleles of the invention ("selectively hybridizable") may  
22 be prepared, and the conditions under which such polynucleotides selectively hybridize with the  
23 polymorphisms of the invention, may be achieved following guidance provided in the art, such  
24 as described by Conner et al., 1983, *Proc. Nat. Acad. Sci. U.S.A.* **80**:278-82; Yershov et al., 1996,  
25 *Proc. Nat. Acad. Sci. U.S.A.* **93**:4913-18; Drobyshev et al., 1997, *Gene* **188**:45-52; and Chee et  
26 al., 1996, *Science* **274**:610-614. Selectively hybridizable reporting polynucleotides such as  
27 molecular beacons are also well known in the art.

28  
29 For preliminary screening for homologous nucleic acids, low stringency hybridization  
30 conditions, corresponding to a  $T_m$  of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% milk, and  
31 no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization  
32 conditions correspond to a higher  $T_m$ , *e.g.*, 40% formamide, with 5x or 6x SSC. High stringency  
33 hybridization conditions correspond to the highest  $T_m$ , *e.g.*, 50% formamide, 5x or 6x SSC.

1 Hybridization requires that the two nucleic acids contain complementary sequences, although  
2 depending on the stringency of the hybridization, mismatches between bases are possible. The  
3 appropriate stringency for selectively hybridizing nucleic acids depends on the length of the  
4 nucleic acids and the degree of complementation, variables well known in the art. The greater  
5 the degree of similarity or homology between two nucleotide sequences, the greater the value of  
6  $T_m$  for hybrids of nucleic acids having those sequences. The relative stability (corresponding to  
7 higher  $T_m$ ) of nucleic acid hybridizations decreases in the following order: RNA:RNA,  
8 DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for  
9 calculating  $T_m$  have been derived (*see* Sambrook et al., *supra*, 9.50-0.51). For hybridization with  
10 shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important,  
11 and the length of the oligonucleotide determines its specificity (*see* Sambrook et al., *supra*, 11.7-  
12 11.8). Preferably a minimum length for a selectively hybridizable nucleic acid is at least about  
13 10 nucleotides; preferably at least about 20 nucleotides; and more preferably the length is at least  
14 about 30 nucleotides; and most preferably 40 nucleotides. As noted above, the skilled artisan  
15 will be guided by the teachings in the art on selecting the length of a polynucleotide or nucleic  
16 acid sequence, the position(s) of the variant nucleotide(s), and the conditions and instrumentation  
17 to selectively identify nucleic acid sequences comprising one or more of the polymorphisms as  
18 described herein.

19  
20 In a specific embodiment, the term "standard hybridization conditions" refers to a  $T_m$  of 55°C,  
21 and utilizes conditions as set forth above. In a preferred embodiment, the  $T_m$  is 60°C; in a more  
22 preferred embodiment, the  $T_m$  is 65°C.

23  
24 "Homologous recombination" refers to the insertion of a foreign DNA sequence of a vector in a  
25 chromosome. Preferably, the vector targets a specific chromosomal site for homologous  
26 recombination. For specific homologous recombination, the vector will contain sufficiently long  
27 regions of homology to sequences of the chromosome to allow complementary binding and  
28 incorporation of the vector into the chromosome. Longer regions of homology, and greater  
29 degrees of sequence similarity, may increase the efficiency of homologous recombination.

30  
31 A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and  
32 translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of  
33 appropriate regulatory sequences. The boundaries of the coding sequence are determined by a

1 start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus.  
2 A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from  
3 eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) DNA, and even  
4 synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell,  
5 a polyadenylation signal and transcription termination sequence will usually be located 3' to the  
6 coding sequence.

7  
8 Transcriptional and translational control sequences are DNA regulatory sequences, such as  
9 promoters, enhancers, terminators, and the like, that provide for the expression of a coding  
10 sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

11  
12 A "promoter sequence" or "promoter" is a DNA regulatory region capable of binding RNA  
13 polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence.  
14 For purposes of defining the present invention, the promoter sequence is bounded at its 3'  
15 terminus by the transcription initiation site and extends upstream (5' direction) to include the  
16 minimum number of bases or elements necessary to initiate transcription at levels detectable  
17 above background. Within the promoter sequence will be found a transcription initiation site  
18 (conveniently defined for example, by mapping with nuclease S1), as well as protein binding  
19 domains (consensus sequences) responsible for the binding of RNA polymerase.

20  
21 A coding sequence is "under the control" of transcriptional and translational control sequences in  
22 a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-  
23 RNA spliced and translated into the protein encoded by the coding sequence.

24  
25 A coding sequence is "operatively associated with" a transcriptional and translational control  
26 sequences, such as a promoter for example, when RNA polymerase transcribes the coding  
27 sequence into mRNA, which in turn is translated into a protein encoding by the coding sequence.

28  
29 A "signal sequence" is included at the beginning of the coding sequence of a protein to be  
30 expressed on the surface of a cell. This sequence encodes a signal peptide, N-terminal to the  
31 mature polypeptide, that directs the host cell to translocate the polypeptide. The term  
32 "translocation signal sequence" is used herein to refer to this sort of signal sequence.  
33 Translocation signal sequences can be found associated with a variety of proteins native to

1 eukaryotes and prokaryotes, and are often functional in both types of organisms.

2  
3 An "expression control sequence" is a DNA sequence that controls and regulates the  
4 transcription and translation of another DNA sequence. A coding sequence is "under the  
5 control" of transcriptional and translational control sequences in a cell when RNA polymerase  
6 transcribes the coding sequence into mRNA, which is then translated into the protein encoded by  
7 the coding sequence.

8  
9 The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a  
10 purified restriction digest or produced synthetically, which is capable of acting as a point of  
11 initiation of synthesis when placed under conditions in which synthesis of a primer extension  
12 product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of  
13 nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and  
14 pH. The primer may be either single-stranded or double-stranded and must be sufficiently long  
15 to prime the synthesis of the desired extension product in the presence of the inducing agent.  
16 The exact length of the primer will depend upon many factors, including temperature, source of  
17 primer and use of the method. For example, for diagnostic applications, depending on the  
18 complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more  
19 nucleotides, although it may contain fewer nucleotides.

20  
21 The primers herein are selected to be "substantially" complementary to different strands of a  
22 particular target DNA sequence. This means that the primers must be sufficiently  
23 complementary to selectively hybridize with their respective strands. Therefore, the primer  
24 sequence need not reflect the exact sequence of the template. For example, a non-  
25 complementary nucleotide fragment may be attached to the 5' end of the primer, with the  
26 remainder of the primer sequence being complementary to the strand. Alternatively, non-  
27 complementary bases or longer sequences can be interspersed into the primer, provided that the  
28 primer sequence has sufficient complementarity with the sequence of the strand to selectively  
29 hybridize therewith and thereby form the template for the synthesis of the extension product.

30  
31 A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been  
32 introduced inside the cell. The transforming DNA may or may not be integrated (covalently  
33 linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and

1 mammalian cells for example, the transforming DNA may be maintained on an episomal  
2 element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in  
3 which the transforming DNA has become integrated into a chromosome so that it is inherited by  
4 daughter cells through chromosome replication. This stability is demonstrated by the ability of  
5 the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells  
6 containing the transforming DNA. A "clone" is a population of cells derived from a single cell  
7 or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of  
8 stable growth *in vitro* for many generations.

9  
10 The phrase "expected to be indicative" is used herein to refer to the correlation between the  
11 identity of the allelic variation(s) in an individual and the susceptibility of an individual to  
12 addictive disease, sensitivity to pain and analgesics, therapeutic effectiveness of analgesics, and  
13 other physiological manifestations described herein related to the function of the kappa opioid  
14 receptor, such as but not limited to the endogenous opioid system, nociception, neurotransmitter  
15 release (including dopamine, GABA, noradrenaline, and serotonin), anxiety and stress, learning,  
16 memory and cognition, alcohol self-administration, behavioral sensitization to cocaine, drug  
17 addiction, opiate withdrawal and tolerance, food intake, immune function, cardiovascular  
18 function, renal function, gastrointestinal function, and motor function. Expected correlations of  
19 kappa opioid receptor alleles and susceptibility to various conditions may be increased  
20 susceptibility or decreased susceptibility.

21  
22 As explained above, within the scope of the present invention are DNA sequences encoding  
23 variant alleles of a human kappa opioid receptor gene of the present invention, which comprise  
24 at least one variation in the predominant or "most common" allele of the human kappa opioid  
25 receptor gene. The most common allele comprises a DNA sequence of SEQ ID NO:1, and  
26 variations in the most common allele comprise G36T, A843G, C846T, C852T, C948T, or  
27 C1008T.

28  
29 As used herein, the term "sequence homology" in all its grammatical forms refers to the  
30 relationship between proteins that possess a "common evolutionary origin," including proteins  
31 from superfamilies (*e.g.*, the immunoglobulin superfamily) and homologous proteins from  
32 different species (*e.g.*, myosin light chain, etc.) (Reeck et al., 1987, *Cell* 50:667).  
33



1 Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of  
2 identity or correspondence between nucleic acid or amino acid sequences of proteins that do not  
3 share a common evolutionary origin (*see* Reeck et al., *supra*). However, in common usage and  
4 in the instant application, the term "homologous," when modified with an adverb such as  
5 "highly," may refer to sequence similarity and not a common evolutionary origin.

6  
7 In a specific embodiment, two DNA sequences are "substantially homologous" or "substantially  
8 similar" when at least about 50% (preferably at least about 75%, and most preferably at least  
9 about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences.  
10 Sequences that are substantially homologous can be identified by comparing the sequences using  
11 standard software available in sequence data banks, or in a Southern hybridization experiment  
12 under, for example, stringent conditions as defined for that particular system. Defining  
13 appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al.,  
14 *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

15  
16 The term "corresponding to" is used herein to refer to similar or homologous sequences, whether  
17 the exact position is identical or different from the molecule to which the similarity or homology  
18 is measured. Thus, the term "corresponding to" refers to the sequence similarity, and not the  
19 numbering of the amino acid residues or nucleotide bases.

20  
21 A variant allele of the human kappa opioid receptor gene of the present invention, whether  
22 genomic DNA or cDNA, can be isolated from any source, particularly from a human cDNA or  
23 genomic library. Methods for obtaining an allele of a human kappa opioid receptor gene,  
24 variants thereof, or the most common, are well known in the art, as described above (*see, e.g.,*  
25 Sambrook et al., 1989, *supra*).

26  
27 Accordingly, any human cell potentially can serve as the nucleic acid source for the molecular  
28 cloning of a variant allele of the human kappa opioid receptor gene of the present invention, or a  
29 nucleic acid molecule selectively hybridizable to a variant allele of a human kappa opioid  
30 receptor gene of the present invention. The DNA may be obtained by standard procedures known  
31 in the art from cloned DNA (*e.g.,* a DNA "library"), and preferably is obtained from a cDNA  
32 library prepared from tissues with high level expression of a human kappa opioid receptor  
33 protein, by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or

1 fragments thereof, purified from the desired cell (See, for example, Sambrook et al., 1989, *supra*;  
2 Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K.  
3 Vol. I, II). Clones derived from genomic DNA may contain regulatory and intron DNA regions  
4 in addition to coding regions; clones derived from cDNA will not contain intron sequences.  
5 Whatever the source, an allele of a human kappa opioid receptor gene of the present invention  
6 should be molecularly cloned into a suitable vector for propagation.

7  
8 In the molecular cloning of a human kappa opioid receptor gene of the present invention, DNA  
9 fragments are generated, some of which will encode an allele. The DNA may be cleaved at  
10 specific sites using various restriction enzymes. Alternatively, one may use DNase in the  
11 presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for  
12 example, by sonication. The linear DNA fragments can then be separated according to size by  
13 standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis  
14 and column chromatography.

15  
16 Once the DNA fragments are generated, identification of the specific DNA fragment containing  
17 an allele of a human kappa opioid receptor of the present invention may be accomplished in a  
18 number of ways. For example, if an amount of a portion of an allele of a human kappa opioid  
19 receptor gene, or its specific RNA, or a fragment thereof, is available and can be purified and  
20 labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the  
21 labeled probe (Benton and Davis, 1977, *Science* **196**:180; Grunstein and Hogness, 1975, *Proc.*  
22 *Natl. Acad. Sci. U.S.A.* **72**:3961). For example, a set of oligonucleotides corresponding to the  
23 partial amino acid sequence information obtained for a human kappa opioid receptor protein can  
24 be prepared and used as probes for DNA encoding a variant allele of a human kappa opioid  
25 receptor gene of the present invention, as was done in a specific example, *infra*, or as primers for  
26 cDNA or mRNA (*e.g.*, in combination with a poly-T primer for RT-PCR). Preferably, a  
27 fragment is selected that is highly unique to a variant allele of the human kappa opioid receptor  
28 gene of the invention. Those DNA fragments with substantial homology to the probe will  
29 selectively hybridize. As noted above, the greater the degree of homology, the more stringent  
30 hybridization conditions can be used.

31  
32 An allele of a human kappa opioid receptor gene of the present invention can also be identified  
33 by mRNA selection, *i.e.*, by nucleic acid hybridization followed by *in vitro* translation. In this

1 procedure, nucleotide fragments are used to isolate complementary mRNAs by hybridization.  
2 Such DNA fragments may represent available, purified DNA of an allele of a human kappa  
3 opioid receptor gene of the present invention, or may be synthetic oligonucleotides designed  
4 from the partial amino acid sequence information. Immunoprecipitation analysis or functional  
5 assays of the *in vitro* translation products of the products of the isolated mRNAs identifies the  
6 mRNA and, therefore, the complementary DNA fragments, that contain the desired sequences.

7  
8 A labeled cDNA of an allele of a human kappa opioid receptor gene of the present invention, or  
9 fragments thereof, or a nucleic acid selectively hybridizable to an allele of a human kappa opioid  
10 receptor gene of the present invention, can be synthesized using sequences set forth herein. The  
11 radiolabeled mRNA or cDNA may then be used as a probe to identify homologous DNA  
12 fragments from among other genomic DNA fragments. Suitable labels include enzymes,  
13 radioactive isotopes, fluorophores (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE),  
14 Texas red (TR), rhodamine, free or chelated lanthanide series salts, especially  $\text{Eu}^{3+}$ , to name a  
15 few fluorophores), chromophores, radioisotopes, chelating agents, dyes, colloidal gold, latex  
16 particles, ligands (e.g., biotin), and chemiluminescent agents. When a control marker is  
17 employed, the same or different labels may be used for the receptor and control marker. As  
18 noted above, molecular beacons capable of identifying the polymorphisms of the invention are  
19 embraced herein.

20  
21 In the instance where a radioactive label, such as the isotopes  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{36}\text{Cl}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{Co}$ ,  
22  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{90}\text{Y}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ , and  $^{186}\text{Re}$  are used, known currently available counting procedures may  
23 be utilized. In the instance where the label is an enzyme, detection may be accomplished by any  
24 of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric,  
25 amperometric or gasometric techniques known in the art.

26  
27 Direct labels are one example of labels which can be used according to the present invention. A  
28 direct label has been defined as an entity, which in its natural state, is readily visible, either to the  
29 naked eye, or with the aid of an optical filter and/or applied stimulation, e.g., U.V. light to  
30 promote fluorescence. Among examples of colored labels, which can be used according to the  
31 present invention, include metallic sol particles, for example, gold sol particles such as those  
32 described by Leuvering (U.S. Patent 4,313,734); dye sol particles such as described by Gribnau  
33 et al. (U.S. Patent 4,373,932) and May et al. (WO 88/08534); dyed latex such as described by

1 May, *supra*, Snyder (EP-a 0 280 559 and 0 281 327); or dyes encapsulated in liposomes as  
2 described by Campbell et al. (U.S. Patent 4,703,017). Other direct labels include a  
3 radionucleotide, a fluorescent moiety or a luminescent moiety. In addition to these direct  
4 labeling devices, indirect labels comprising enzymes can also be used according to the present  
5 invention. Various types of enzyme linked immunoassays are well known in the art, for  
6 example, alkaline phosphatase and horseradish peroxidase, lysozyme, glucose-6-phosphate  
7 dehydrogenase, lactate dehydrogenase, urease, these and others have been discussed in detail by  
8 Eva Engvall in Enzyme Immunoassay ELISA and EMIT in *Methods in Enzymology*, 70. 419-  
9 439, 1980 and in U.S. Patent 4,857,453.

10  
11 Other labels for use in the invention include magnetic beads or magnetic resonance imaging  
12 labels.

#### 13 14 Cloning Vectors

15 The present invention also relates to cloning vectors comprising variant alleles of a human kappa  
16 opioid receptor gene of the present invention, and an origin of replication. For purposes of this  
17 Application, an "origin of replication refers to those DNA sequences that participate in DNA  
18 synthesis.

19  
20 As explained above, in an embodiment of the present invention, variant alleles of a human kappa  
21 opioid receptor gene of the present invention comprise a DNA sequence having at least one  
22 variation in the most common allele of a human kappa opioid receptor gene comprising a DNA  
23 sequence of SEQ ID NO:1, wherein the variation comprises G36T, A843G, C846T, C852T,  
24 C948T, or C1008T, or combinations thereof.

25  
26 Furthermore, an isolated variant allele of a human kappa opioid receptor gene of the present  
27 invention, or isolated nucleic acid molecules selectively hybridizable to an isolated variant allele  
28 of a human kappa opioid receptor gene of the present invention, can be inserted into an  
29 appropriate cloning vector in order to produce multiple copies of the variant allele or isolated  
30 nucleic acid molecule. A large number of vector-host systems known in the art may be used.  
31 Possible vectors include, but are not limited to, plasmids or modified viruses. The vector system  
32 used however must be compatible with the host cell used. Examples of vectors include having  
33 applications herein, but are not limited to *E. coli*, bacteriophages such as lambda derivatives, or

1 plasmids such as pBR322 derivatives or pUC plasmid derivatives, e.g., pGEX vectors, pmal-c,  
2 pFLAG, etc. The insertion into a cloning vector can, for example, be accomplished by ligating a  
3 variant allele of the human kappa opioid receptor gene of the present invention, or an isolated  
4 nucleic acid selectively hybridizable thereto, into a cloning vector which has complementary  
5 cohesive termini. However, if the complementary restriction sites used to fragment the variant  
6 allele or isolated nucleic acid selectively hybridizable thereto are not present in the cloning  
7 vector, the ends of the variant allele or the isolated nucleic acid molecule selectively  
8 hybridizable thereto may be enzymatically modified. Alternatively, any site desired may be  
9 produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers  
10 may comprise specific chemically synthesized oligonucleotides encoding restriction  
11 endonuclease recognition sequences. Such recombinant molecules can then be introduced into  
12 host cells via transformation, transfection, infection, electroporation, etc., so that many copies of  
13 a variant allele of a human kappa opioid receptor gene of the present invention, or an isolated  
14 nucleic acid molecule selectively hybridizable thereto, can be generated. Preferably, the cloned  
15 isolated variant is contained on a shuttle vector plasmid, which provides for expansion in a  
16 cloning cell, e.g., *E. coli*, and facile purification for subsequent insertion into an appropriate  
17 expression cell line, if such is desired. For example, a shuttle vector, which is a vector that can  
18 replicate in more than one type of organism, can be prepared for replication in both *E. coli* and  
19 *Saccharomyces cerevisiae* by linking sequences from an *E. coli* plasmid with sequences from the  
20 yeast 2μ plasmid.

21  
22 In an alternative method an isolated variant allele of a human kappa opioid receptor gene of the  
23 present invention or an isolated nucleic acid molecule selectively hybridizable thereto may be  
24 identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach.  
25 Enrichment for a variant allele, for example, by size fractionation, can be done before insertion  
26 into the cloning vector.

### 27 28 Expression Vectors

29 As stated above, the present invention extends to an isolated variant allele of a human kappa  
30 opioid receptor gene, comprising a DNA sequence having at least one variation in the DNA  
31 sequence of the predominant or "most common" allele of the human kappa opioid receptor gene  
32 comprising a DNA sequence of SEQ ID NO:1 wherein the variations comprise G36T, A843G,  
33 C846T, C852T, C948T, or C1008T, or combinations thereof.

1 Variant alleles of the present invention, along with isolated nucleic acid molecules selectively  
2 hybridizable to such variant alleles, can be inserted into an appropriate expression vector, *i.e.*, a  
3 vector which contains the necessary elements for the transcription and translation of the inserted  
4 protein-coding sequence. Thus, a variant allele of the present invention, or an isolated nucleic  
5 acid molecule selectively hybridizable to a variant allele of the present invention, is operatively  
6 associated with a promoter in an expression vector of the invention. A DNA sequence is  
7 "operatively associated" to an expression control sequence, such as a promoter, when the  
8 expression control sequence controls and regulates the transcription and translation of that DNA  
9 sequence. The term "operatively associated" includes having an appropriate start signal (e.g.,  
10 ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to  
11 permit expression of the DNA sequence under the control of the expression control sequence and  
12 production of the desired product encoded by the DNA sequence. If a variant allele of the  
13 present invention, or an isolated nucleic acid selectively hybridizable thereto does not contain an  
14 appropriate start signal, such a start signal can be inserted into the expression vector in front of  
15 (5' of) the molecule.

16  
17 Both cDNA and genomic sequences can be cloned and expressed under control of such  
18 regulatory sequences. An expression vector also preferably includes a replication origin.

19  
20 The necessary transcriptional and translational signals can be provided on a recombinant  
21 expression vector, or they may be supplied by an allele comprising a human kappa opioid  
22 receptor gene.  
23

24 Potential host-vector systems include but are not limited to mammalian cell systems infected  
25 with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*,  
26 baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed  
27 with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors  
28 vary in their strengths and specificities. Depending on the host-vector system utilized, any one  
29 of a number of suitable transcription and translation elements may be used.  
30

31 A variant allele of a human kappa opioid receptor gene of the present invention or an isolated  
32 nucleic acid molecule selectively hybridizable thereto may be expressed chromosomally, after  
33 integration of the coding sequence by recombination. In this regard, any of a number of

1 amplification systems may be used to achieve high levels of stable gene expression (See  
2 Sambrook et al., 1989, *supra*).  
3

4 A unicellular host transformed or transfected with an expression vector of the present invention  
5 is cultured in an appropriate cell culture medium that provides for expression by the unicellular  
6 host of the variant allele, or isolated nucleic acid selectively hybridizable thereto.  
7

8 Any of the methods previously described for the insertion of DNA fragments into a cloning  
9 vector may be used to construct expression vectors of the present invention. These methods may  
10 include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombination (genetic  
11 recombination).  
12

13 Expression of a variant allele of a human kappa opioid receptor gene of the present invention or  
14 an isolated nucleic acid molecule selectively hybridizable to a variant allele of a human kappa  
15 opioid receptor gene, may be controlled by any promoter/enhancer element known in the art, but  
16 these regulatory elements must be functional in the host selected for expression. Promoters  
17 which may be used to control expression include, but are not limited to, the SV40 early promoter  
18 region (Benoist and Chambon, 1981, *Nature* **290**:304-310), the promoter contained in the 3' long  
19 terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, *Cell* 22:787-797), the herpes  
20 thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445),  
21 the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42);  
22 prokaryotic expression vectors such as the  $\beta$ -lactamase promoter (Villa-Kamaroff, et al., 1978,  
23 *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, *Proc.*  
24 *Natl. Acad. Sci. U.S.A.* 80:21-25); see also "Useful proteins from recombinant bacteria" in  
25 *Scientific American*, 1980, 242:74-94; promoter elements from yeast or other fungi such as the  
26 Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase)  
27 promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which  
28 exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control  
29 region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al.,  
30 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology*  
31 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985,  
32 *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells  
33 (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander

1 et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is  
2 active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495),  
3 albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel.  
4 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985,  
5 Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58), alpha 1-antitrypsin gene  
6 control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171),  
7 beta-globin gene control region which is active in myeloid cells (Mogam et al., 1985, Nature  
8 315:338-340; Kollias et al., 1986, Cell 46:89-94), myelin basic protein gene control region which  
9 is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712), myosin  
10 light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-  
11 286), and gonadal releasing hormone gene control region which is active in the hypothalamus  
12 (Mason et al., 1986, Science 234:1372-1378).

13 Moreover, expression vectors comprising a variant allele of a human kappa opioid receptor gene  
14 of the present invention, or an isolated nucleic acid molecule selectively hybridizable thereto,  
15 can be identified by four general approaches: (a) PCR amplification of the desired plasmid DNA  
16 or specific mRNA, (b) nucleic acid hybridization, (c) presence or absence of selection marker  
17 gene functions, and (d) expression of inserted sequences. In the first approach, the variant allele  
18 or isolated nucleic acid molecule selectively hybridizable thereto can be amplified by PCR to  
19 provide for detection of the amplified product. This includes a molecular beacon approach to  
20 identifying the polymorphisms herein. In the second approach, the presence of a foreign gene  
21 inserted into an expression vector of the present invention can be detected by nucleic acid  
22 hybridization using probes comprising sequences that are homologous to an inserted marker  
23 gene. In the third approach, the recombinant vector/host system can be identified and selected  
24 based upon the presence or absence of certain "selection marker" gene functions (*e.g.*,  $\beta$ -  
25 galactosidase activity, thymidine kinase activity, resistance to antibiotics, transformation  
26 phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign  
27 genes in the vector. In yet another example, if an isolated variant allele of a human kappa opioid  
28 receptor gene of the present invention, or an isolated nucleic acid molecule selectively  
29 hybridizable thereto, is inserted within the "selection marker" gene sequence of the vector,  
30 recombinants containing the insert can be identified by the absence of the inserted gene function.  
31 In the fourth approach, recombinant expression vectors can be identified by assaying for the  
32 activity, biochemical, or immunological characteristics of the gene product expressed by the  
33



1 recombinant, provided that the expressed protein assumes a functionally active conformation.

2  
3 Naturally, the present invention extends to a method of producing a human kappa opioid receptor  
4 from the polymorphic variants described herein. Although the variants described herein are  
5 "silent," as they do not alter the amino acid sequence of the kappa opioid gene product (i.e., the  
6 receptor), the methods herein may be used to determine altered levels of gene expression as a  
7 consequence of the presence of one or more of the polymorphisms described herein. An  
8 example of such a method comprises the steps of culturing a unicellular host transformed or  
9 transfected with an expression vector comprising a variant allele of a human kappa opioid  
10 receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the  
11 variant allele which is operatively associated with a promoter. The transformed or transfected  
12 unicellular host is then cultured under conditions that provide for expression of the variant allele  
13 of the human kappa opioid receptor gene, and the expression product is recovered from the  
14 unicellular host.

15  
16 Another example involves culturing a unicellular host transformed or transfected with an isolated  
17 nucleic acid molecule selectively hybridizable to a variant allele of a human kappa opioid  
18 receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1,  
19 wherein the isolated nucleic acid molecule is operatively associated with a promoter. The  
20 variant human kappa opioid receptor is then recovered from the host.

21  
22 A wide variety of unicellular host/expression vector combinations may be employed in  
23 expressing the DNA sequences of this invention. Useful expression vectors, for example, may  
24 consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable  
25 vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col El,  
26 pCR1, pBR322, pMal-C2, pET, pGEX (Smith *et al.*, 1988, Gene 67:31-40), pMB9 and their  
27 derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage  $\lambda$ , e.g.,  
28 NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast  
29 plasmids such as the 2 $\mu$  plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as  
30 vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and  
31 phage DNAs, such as plasmids that have been modified to employ phage DNA or other  
32 expression control sequences; and the like.  
33

1 For example, in a baculovirus expression systems, both non-fusion transfer vectors, such as but  
2 not limited to pVL941 (*Bam*H1 cloning site; Summers), pVL1393 (*Bam*H1, *Sma*I, *Xba*I, *Eco*R1,  
3 *Not*I, *Xma*III, *Bgl*II, and *Pst*I cloning site; Invitrogen), pVL1392 (*Bgl*II, *Pst*I, *Not*I, *Xma*III,  
4 *Eco*R1, *Xba*I, *Sma*I, and *Bam*H1 cloning site; Summers and Invitrogen), and pBlueBacIII  
5 (*Bam*H1, *Bgl*II, *Pst*I, *Nco*I, and *Hind*III cloning site, with blue/white recombinant screening  
6 possible; Invitrogen), and fusion transfer vectors, such as but not limited to pAc700 (*Bam*H1 and  
7 *Kpn*I cloning site, in which the *Bam*H1 recognition site begins with the initiation codon;  
8 Summers), pAc701 and pAc702 (same as pAc700, with different reading frames), pAc360  
9 (*Bam*H1 cloning site 36 base pairs downstream of a polyhedrin initiation codon;  
10 Invitrogen(195)), and pBlueBacHisA, B, C (three different reading frames, with *Bam*H1, *Bgl*II,  
11 *Pst*I, *Nco*I, and *Hind*III cloning site, an N-terminal peptide for ProBond purification, and  
12 blue/white recombinant screening of plaques; Invitrogen (220)) can be used.

13  
14 Mammalian expression vectors contemplated for use in the invention include vectors with  
15 inducible promoters, such as the dihydrofolate reductase (DHFR) promoter, *e.g.*, any expression  
16 vector with a *DHFR* expression vector, or a *DHFR*/methotrexate co-amplification vector, such as  
17 pED *Pst*I, *Sal*I, *Sba*I, *Sma*I, and *Eco*RI cloning site, with the vector expressing both the cloned  
18 gene and *DHFR*; *see* Kaufman, *Current Protocols in Molecular Biology*, 16.12 (1991).

19  
20 Alternatively, a glutamine synthetase/methionine sulfoximine co-amplification vector, such as  
21 pEE14 (*Hind*III, *Xba*I, *Sma*I, *Sba*I, *Eco*RI, and *Bcl*I cloning site, in which the vector expresses  
22 glutamine synthase and the cloned gene; Celltech). In another embodiment, a vector that directs  
23 episomal expression under control of Epstein Barr Virus (EBV) can be used, such as pREP4  
24 (*Bam*H1, *Sfi*I, *Xho*I, *Not*I, *Nhe*I, *Hind*III, *Nhe*I, *Pvu*II, and *Kpn*I cloning site, constitutive RSV-  
25 LTR promoter, hygromycin selectable marker; Invitrogen), pCEP4 (*Bam*H1, *Sfi*I, *Xho*I, *Not*I,  
26 *Nhe*I, *Hind*III, *Nhe*I, *Pvu*II, and *Kpn*I cloning site, constitutive hCMV immediate early gene,  
27 hygromycin selectable marker; Invitrogen), pMEP4 (*Kpn*I, *Pvu*I, *Nhe*I, *Hind*III, *Not*I, *Xho*I, *Sfi*I,  
28 *Bam*H1 cloning site, inducible metallothionein IIa gene promoter, hygromycin selectable  
29 marker; Invitrogen), pREP8 (*Bam*H1, *Xho*I, *Not*I, *Hind*III, *Nhe*I, and *Kpn*I cloning site, RSV-  
30 LTR promoter, histidinol selectable marker; Invitrogen), pREP9 (*Kpn*I, *Nhe*I, *Hind*III, *Not*I,  
31 *Xho*I, *Sfi*I, and *Bam*HI cloning site, RSV-LTR promoter, G418 selectable marker; Invitrogen),  
32 and pEBVHis (RSV-LTR promoter, hygromycin selectable marker, N-terminal peptide  
33 purifiable via ProBond resin and cleaved by enterokinase; Invitrogen). Selectable mammalian

1 expression vectors for use in the invention include pRc/CMV (*HindIII*, *BstXI*, *NotI*, *SbaI*, and  
2 *ApaI* cloning site, G418 selection; Invitrogen), pRc/RSV (*HindIII*, *SpeI*, *BstXI*, *NotI*, *XbaI*  
3 cloning site, G418 selection; Invitrogen), and others. Vaccinia virus mammalian expression  
4 vectors (see, Kaufman, 1991, *supra*) for use according to the invention include but are not  
5 limited to pSC11 (*SmaI* cloning site, TK- and  $\beta$ -gal selection), pMJ601 (*SalI*, *SmaI*, *AflI*, *NarI*,  
6 *BspMII*, *BamHI*, *ApaI*, *NheI*, *SacII*, *KpnI*, and *HindIII* cloning site; TK- and  $\beta$ -gal selection), and  
7 pTKgptF1S (*EcoRI*, *PstI*, *SalI*, *AccI*, *HindII*, *SbaI*, *BamHI*, and Hpa cloning site, TK or XPRT  
8 selection).

9  
10 Yeast expression systems can also be used according to the invention to produce a variant human  
11 kappa opioid receptor or the present invention. For example, the non-fusion pYES2 vector  
12 (*XbaI*, *SphI*, *ShoI*, *NotI*, *GstXI*, *EcoRI*, *BstXI*, *BamHI*, *SacI*, *KpnI*, and *HindIII* cloning sit;  
13 Invitrogen) or the fusion pYESHisA, B, C (*XbaI*, *SphI*, *ShoI*, *NotI*, *BstXI*, *EcoRI*, *BamHI*, *SacI*,  
14 *KpnI*, and *HindIII* cloning site, N-terminal peptide purified with ProBond resin and cleaved with  
15 enterokinase; Invitrogen), to mention just two, can be employed according to the invention.

16  
17 Once a particular recombinant DNA molecule is identified and isolated, several methods known  
18 in the art may be used to propagate it. Once a suitable host system and growth conditions are  
19 established, recombinant expression vectors can be propagated and prepared in quantity. As  
20 previously explained, the expression vectors which can be used include, but are not limited to the  
21 following vectors or their derivatives: human or animal viruses such as vaccinia virus or  
22 adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g.,  
23 lambda), and plasmid and cosmid DNA vectors, to name but a few.

24  
25 Examples of unicellular hosts contemplated by the present invention include, but are not limited  
26 to *E. coli* Pseudomonas, Bacillus, Streptomyces, yeast, CHO, R1.1, B-W, L-M, COS1, COS7,  
27 BSC1, BSC40, BMT10 and Sf9 cells. In addition, a host cell strain may be chosen which  
28 modulates the expression of a variant allele comprising a human kappa opioid receptor gene, or  
29 an isolated nucleic acid selectively hybridizable thereto, such that the gene product is modified  
30 and processed in the specific fashion desired. Different host cells have characteristic and  
31 specific mechanisms for the translational and post-translational processing and modification  
32 (e.g., glycosylation, cleavage [e.g., of signal sequence]) of proteins. Appropriate cell lines or  
33 host systems can be chosen to ensure the desired modification and processing of the foreign

1 protein expressed. For example, expression in a bacterial system can be used to produce an  
2 nonglycosylated core protein product. However, a translocation signal sequence of an isolated  
3 variant allele of a human kappa opioid receptor gene of the present invention, or an isolated  
4 nucleic acid selectively hybridizable thereto, expressed in bacteria may not be properly spliced.  
5 Expression in yeast can produce a glycosylated product. Expression in eukaryotic cells can  
6 increase the likelihood of "native" glycosylation and folding. Moreover, expression in  
7 mammalian cells can provide a tool for reconstituting, or constituting activity of the variant  
8 human kappa opioid receptor gene. Furthermore, different vector/host expression systems may  
9 affect processing reactions, such as proteolytic cleavages, to a different extent.

10  
11 Vectors are introduced into the desired unicellular hosts by methods known in the art, *e.g.*,  
12 transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium  
13 phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector  
14 transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol.  
15 Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed  
16 March 15, 1990).

17  
18 Consequently, the present invention extends to a method for determining a susceptibility of a  
19 subject to a disease comprising removing a bodily sample comprising a first and second allele of  
20 a human kappa opioid receptor gene from the subject, and determining whether either the first or  
21 second alleles, or both alleles comprise a DNA sequence having at least one variation in SEQ ID  
22 NO:1, wherein the variation comprises G36T, A843G, C846T, C852T, C948T, or C1008T.

23  
24 Variant alleles of a human kappa opioid receptor gene indicating increased or decrease  
25 susceptibility to diseases in the subject as described above, can be detected from cellular sources,  
26 such as, but not limited to, whole blood, epithelial cells obtained from the mouth, brain tissue  
27 biopsies, adipocytes, testes, heart, and the like. For example, cells can be obtained from an  
28 individual by biopsy and lysed, *e.g.*, by freeze-thaw cycling, or treatment with a mild cytolytic  
29 detergent such as, but not limited to, TRITON X-100®, digitonin, NONIDET P (NP)-40®,  
30 saponin, and the like, or combinations thereof (see, *e.g.*, International Patent Publication WO  
31 92/08981, published May 29, 1992). In yet another embodiment, samples containing both cells  
32 and body fluids can be used (see *ibid.*).

1 Other methods presently understood by a skilled artisan, and encompassed by the present  
2 invention, can also be used to detect the presence of either variation in either or both alleles of a  
3 human kappa opioid receptor gene in a sample, and hence increased or decreased susceptibility  
4 to at least one disease of the subject relative to the susceptibility of at least one disease in a  
5 standard comprising alleles of the human kappa opioid receptor gene comprising a DNA  
6 sequence of SEQ ID NO:1.

7  
8 For example, an optionally detectably labeled isolated nucleic acid molecule selectively  
9 hybridizable to an isolated variant allele of a human kappa opioid receptor gene comprising a  
10 DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises G36T,  
11 A843G, C846T, C852T, C948T, or C1008T, can be used in standard Northern hybridization  
12 analysis to detect the presence, and in some instances quantitate the level of transcription of such  
13 a variant allele of the present invention.

14  
15 Alternatively, oligonucleotides of the invention can be used as PCR primers to amplify an allele  
16 of a human kappa opioid receptor gene of the biological sample *e.g.*, by reverse transcriptase-  
17 PCR, or amplification of the allele itself. The amplified mRNA or DNA can then be quantified  
18 or sequenced in order to determine the presence of a variant allele, and the susceptibility of the  
19 subject to addictive diseases. Furthermore, variations in SEQ ID NO:1, as described above, can  
20 be found by creation or deletion of restriction fragment length polymorphisms (RFLPs) not  
21 found in the predominant or "most common" allele, hybridization with a specific probe  
22 engineered to selectively hybridize to variation described, (or lack of hybridization with a probe  
23 specific for the predominant or "most common" allele), as well as by other techniques.

24  
25 Furthermore, biochemical or immunochemical/biochemical (*e.g.*, immunoprecipitation)  
26 techniques can be used to detect the presence and or level of expression of a variant allele of a  
27 human kappa opioid receptor gene comprising a DNA sequence having a variation in SEQ ID  
28 NO:1 as described herein.

#### 30 Determining susceptibility to pain in a Subject

31 In yet another embodiment, the present invention extends to a method for determining a  
32 susceptibility to pain in a subject.  
33

1 Hence, disclosed herein is a method of determining susceptibility of pain in a subject,  
2 comprising the steps of removing a bodily sample comprising a first and second allele of a  
3 human kappa opioid receptor gene from the subject, and determining whether either the first or  
4 second alleles, or both alleles, comprise a DNA sequence having at least one variation in SEQ ID  
5 NO:1, wherein the variation comprises G36T, A843G, C846T, C852T, C948T, or C1008T.  
6

7  
8 The presence of at least one variation in either or both alleles of the human kappa opioid receptor  
9 gene is expected to be indicative of the subject's increased or decreased susceptibility to pain  
10 relative to a person homozygous with respect to the predominant or "most common" allele  
11 comprising a human kappa opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.  
12

13 Numerous methods presently available, and understood by the skilled artisan, can be used to  
14 "genotype" a subject in regards to the presence of a variant allele of a human kappa opioid  
15 receptor gene in the genome of the subject. In particular, methods described above to ascertain  
16 increased or decreased susceptibility to addictive diseases have relevance in this embodiment of  
17 the present invention, and can readily be used herein. For example, Northern blot hybridization  
18 an isolated nucleic acid of the present invention selectively hybridizable to an isolated variant  
19 allele of a human kappa opioid receptor gene comprising a DNA sequence having a variation of  
20 SEQ ID NO:1, wherein the variation comprises G36T, A843G, C846T, C852T, C948T, or  
21 C1008T, as a probe, along with RT-PCR, PCR, and numerous immunoassays described above,  
22 have applications herein.  
23

24 Moreover, once susceptibility to pain in a subject has been determined, it is possible for  
25 attending medical professionals treating the subject for pain to administer an appropriate amount  
26 of pain reliever to the subject in order to induce analgesia. More specifically, an inappropriate  
27 amount of pain reliever is administered to a subject when either the subject is not relieved of  
28 pain, or the subject is exposed to potential deleterious side effects of the pain reliever, such as  
29 induction of addiction to the pain reliever, brain damage, or death.  
30

31 However, since the amount of pain reliever administered to a subject is presently based  
32 principally on weight, information regarding the genotype of the subject with respect to the  
33 human kappa opioid receptor gene can help increase accuracy in determining a therapeutically

1 effective amount of pain reliever to administer in order to induce analgesia, making the use of  
2 pain relievers much safer for the subject.

3  
4 Similarly, once ascertained, a susceptibility to addiction and response to human kappa opioid  
5 receptor directed therapeutic agents, appropriate medications and dosages thereof can be  
6 determined for treatment of addictive diseases.

#### 7 8 Commercial Kits

9 Furthermore, as explained above, the present invention extends to commercial kits having  
10 applications in screening a bodily sample comprising DNA or RNA taken from a subject for the  
11 presence of a variant allele comprising a human kappa opioid receptor comprising a DNA  
12 sequence having a variation in SEQ ID NO:1, wherein the variation comprises G36T, A843G,  
13 C846T, C852T, C948T, or C1008T, or combinations thereof.

14  
15 With information obtained from the use of a test kit of the present invention, an attending health  
16 profession can determine whether the subject has an susceptibility to pain relative to a standard,  
17 an increased susceptibility to at least one addictive disease relative to the susceptibility of a  
18 standard, a therapeutically effective amount of pain reliever to administer to the subject suffering  
19 from pain in order to induce analgesia in the subject relative to the therapeutically effective  
20 amount of pain reliever to administer to a standard in order to induce analgesia in the standard,  
21 or a therapeutically effective amount therapeutic agent to administer to a subject suffering from  
22 at least one addictive disease, relative to the therapeutically effective amount of therapeutic agent  
23 to administer to standard suffering from at least one addictive disease. Furthermore, such  
24 information can also be used to diagnose a disease or disorder related to a physiological function  
25 of the endogenous opioid system, nociception, neurotransmitter release (including dopamine,  
26 GABA, noradrenaline, and serotonin), anxiety and stress, learning, memory and cognition,  
27 alcohol self-administration, behavioral sensitization to cocaine, drug addition, opiate withdrawal  
28 and tolerance, food intake, immune function, cardiovascular function, renal function,  
29 gastrointestinal function, and motor function. In each use described above, the standard  
30 comprises a first and or second allele of a human kappa opioid receptor gene comprising a DNA  
31 sequence of SEQ ID NO:1.

32  
33 Accordingly, a test kit of the present invention for determining whether a subject comprises a

1 variant allele of a human kappa opioid receptor gene comprising a DNA sequence having a  
2 variation in SEQ ID NO:1, comprises means for detecting the presence of a variation in a first  
3 and or second allele comprising a human kappa opioid receptor in a biological sample from a  
4 subject, and optimally packaged with directions for use of the kit. In one particular aspect, a test  
5 kit comprises an oligonucleotide probe(s) for binding to a variant allele of a human kappa opioid  
6 receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1; and means for  
7 detecting the level of binding of the probe to the variant allele, wherein detection binding of the  
8 probe to the variant allele indicates the presence of a variant comprising a human kappa opioid  
9 receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the  
10 variation comprises G36T, A843G, C846T, C852T, C948T, or C1008T, or combinations thereof.

11  
12 The sequence of the oligonucleotide probe used in a commercial kit will determine which if any  
13 variation is present in an allele comprising a human kappa opioid receptor gene. Should no  
14 binding be detected, it is probable that no such variation exists in either allele of the subject.

15  
16 More specifically, a commercial test kit of the present invention comprises:

- 17 a) PCR oligonucleotide primers suitable for detection of a variant allele of a  
18 human kappa opioid receptor gene comprising a DNA sequence having a  
19 variation in SEQ ID NO:1, as set forth above,  
20 b) other reagents; and  
21 c) directions for use of the kit.

22  
23 Examples of PCR oligonucleotide primer suitable for detection of an allele comprising a human  
24 kappa opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1 can  
25 be readily produced by a person of ordinary skill in the art with teaching set forth herein, and  
26 variations of SEQ ID NO:1 also set forth herein.

27  
28  
29 The present invention may be better understood by reference to the following non-limiting  
30 Example, which is provided as exemplary of the invention. The following Example is presented  
31 in order to more fully illustrate the preferred embodiments of the invention. It should in no way  
32 be construed, however, as limiting the broad scope of the invention.



## EXAMPLE

To identify polymorphisms of the human kappa opioid receptor, a PCR-based strategy was used to amplify the coding regions of the kappa opioid receptor gene, and to determine the DNA sequence of the amplified exons. Using this method DNA samples were sequenced from 189 unrelated subjects.

Study subjects and procedures. Addictive disease patients, specifically long-term heroin addicts currently in chronic methadone maintenance treatment, and normal control subjects with no history of any drug or alcohol abuse, and individuals with non-opiate drug abuse and dependence were extensively characterized with respect to drug abuse, the addictive diseases, psychological and psychiatric profiles, and medical and ethnic family backgrounds. Unrelated study subjects who were former heroin addicts were referred from methadone treatment clinics in the greater New York City area, primarily those associated with The Biology of Addictive Diseases Laboratory located at The Rockefeller University. These clinics are the Adolescent Development Program and Adult Clinic at the New York Hospital-Cornell Medical Center. Previously heroin-addicted patients admitted to the study conformed to the federally regulated criteria for admission to a methadone maintenance program, that is, one or more years of daily multiple-dose self-administration of heroin or other opiates with the development of tolerance, dependence, and drug-seeking behavior. Current or prior abuse of other drugs was not used as an exclusion criterion for this group as long as opioid abuse continued to be the primary diagnosis.

Unrelated healthy volunteer subjects were recruited primarily through posting of notices and newspaper advertisements or referral by physicians or staff at the Rockefeller University Hospital. Individuals with continuing drug or alcohol abuse or prior extended periods of regular abuse were also studied.

Both addictive disease patients and normal volunteers admitted to the study were assessed by a psychiatrist or research nurse with several psychiatric and psychological instruments as well as the Addiction Severity Index. Study subjects were also administered a detailed personal and medical and special addictive disease questionnaire as well as a family history medical and

1 addictive disease questionnaire designed to provide information regarding substance abuse and  
2 major mental illness of first and second degree relatives. Study subjects provided detailed  
3 information regarding family origin and ethnic background, including country or geographic area  
4 of birth. This information was obtained for both the study subjects themselves and their  
5 immediate ancestors (parents, grandparents and great-grandparents), to the extent that the  
6 information was known by the study subjects. Study subjects were classified into five groups:  
7 African-American, Caucasian, Hispanic (Caribbean and Central or South American origin),  
8 Native North American, and Other. The detailed ancestral information collected by the family  
9 origin questionnaire allowed classification of study subjects into defined categories. Following  
10 psychiatric and behavioral assessment and informed consent and family history acquisition,  
11 venipuncture on the study subject was performed, and a blood specimen was taken. Blood  
12 samples were processed for DNA extraction and EBV transformation to create stable cell lines  
13 that were stored for future studies. All blood samples were coded; the psychiatrists and nurses  
14 who performed psychiatric and psychological assessments were blind to the genotypes of the  
15 study subjects, and the identity and categorization of the study subjects was unknown to the  
16 laboratory research personnel.

17  
18 By sequencing PCR-amplified DNA from the study subjects, the most common or wild-type  
19 allele of the hKOR gene (SEQ ID No:1) was identified, and it was determined that the  
20 previously-known GenBank sequence NM\_000912, comprised three polymorphic alleles, as  
21 compared to the GenBank sequences U17298 and L37362. Based on this limited data, it was not  
22 until the present inventors sequenced a large number of hKOR alleles that the most common, or  
23 wild-type, allele was confirmable, and the three variations in the NM\_000912 identified as  
24 polymorphisms and not as suspected sequencing errors. The polymorphisms presently  
25 recognized in the NM\_000912 sequence were confirmed by finding patients in the present study  
26 population with such polymorphisms.

27  
28 Moreover, three further single-nucleotide polymorphisms were identified among the study  
29 subjects. These SNPs were identified in a cohort of 61 (Exon I) and of 189 (Exon III) study  
30 subjects. Many of the subjects had multiple variant allelic forms in exon III: six subjects have  
31 three SNPs and 19 subjects have two SNPs with different constellations. For SNPs in the  
32 predicted mRNA sequence the number +1 is assigned to the first base of the ATG start codon of  
33 the receptor. The polymorphisms identified herein are C852T (SEQ ID No:2), present in

transmembrane region (TM) VI of exon III; C948T (SEQ ID No:3), present in TM VII of exon III; C1008T (SEQ ID No:4), present in the C-terminal region of exon III; G36T (SEQ ID No:5), present in the N-terminal portion of exon I; A843G (SEQ ID No:6), present in TM VI of exon III; and C846T (SEQ ID No:7), present in TM VI of exon III.

The polymorphisms and number of individuals in which they were identified are as follows:

<u>Variant</u>	<u>Location</u>	<u>Position</u>	<u># of individuals</u>	<u>Allelic Freq. of variant</u> <u>SNP</u>
G36T	Exon I	N-terminal	n=61, 14 heterozygous G/T 4 homozygous T/T	18.0%
A843G	Exon III	TM VI	n=189, 53 heterozygous A/G 22 homozygous G/G	25.7%
C846T	Exon III	TM VI	n=189, 24 heterozygous, C/T 2 homozygous T/T	7.4%
C852T	Exon III	TM VI	n=189, 1 heterozygous C/T	< 1%
C948T	Exon III	TM VII	n=189, 6 heterozygous C/T	1.6%
C1008T	Exon III	C-terminal	n=189, 2 heterozygous C/T	< 1%

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.